

ENHANCED ANTIGEN PRESENTING ABILITY OF RNA CAR T CELLS BY CO-INTRODUCTION OF COSTIMULATORY MOLECULES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 15/113,044, filed Jul. 20, 2016, which is a national stage application under 35 U.S.C. § 371 of International Application No. PCT/US2015/012284, filed Jan. 21, 2015, published as International Publication No. WO2015/112626 on Jul. 30, 2015, which application claims priority to U.S. Ser. No. 61/929,813, filed Jan. 21, 2014, the entire contents of each of which are incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 17, 2015, is named N2067-7036WO_SL.TXT and is 76,949 bytes in size.

BACKGROUND

[0003] Chimeric antigen receptors (CARs) are molecules that combine antibody-based specificity for disease-associated surface antigens with T cell receptor-activating intracellular domains with disease-directed cellular immune activity. This configuration allows T cells engineered to express a CAR to achieve MHC-independent primary activation through single chain Fv (scFv) antigen-specific extracellular regions fused to intracellular domains that provide T cell activation and co-stimulatory signals. Second and third generation CARs also provide appropriate co-stimulatory signals via CD28 and/or CD 137 (4-1BB) intracellular activation motifs, which augment cytokine secretion and anti-tumor activity in a variety of solid tumor and leukemia models (Pinthus, et al, 2004, J Clin Invest 114(12): 1774-1781; Milone, et al. 2009, Mol Ther 17(8): 1453-1464; Sadelain, et al, 2009, Curr Opin Immunol 21(2):215-223). The benefit of bypassing the need for antigen presentation by MHC molecules to achieve cytotoxicity makes CAR-engineered T cells an attractive therapeutic modality.

[0004] Adoptive transfer of cytotoxic T lymphocytes (CTLs) has shown great promise in both viral and cancer indications. After many years of less than optimum results with CAR-based T-cell therapy, improved culture systems and cell engineering technologies have made possible CAR T cells with more potent antitumor effects (Sadelain et al, 2009, Curr Opin Immunol 21:215-23). The technology has also been successfully applied in the clinical context, with improved clinical results being reported for CARs introduced with retroviral vectors (Till et al. 2008, Blood 112: 2261-71; Pule et al, 2008, Nat Med 14: 1264-70). These CAR T cells also exhibit enhanced toxicity (Brentjens et al 2010, Mol Ther 18:666-8; Morgan et al, 2010, Mol Ther 18:843-51).

[0005] As an emerging technology, there is an urgent need in the art for improving on existing CAR-based therapies that would allow for more effective, safe, and efficient adoptive transfer of CTLs.

SUMMARY

[0006] The present invention provides T cells engineered to exhibit increased anti-tumor activity by co-expressing a chimeric antigen receptor (CAR) and one or more enhancers of T cell priming (hereafter “ETPs”). The addition of an ETP component to the CAR T cell confers enhanced “professional” antigen-presenting cell (APC) function, which confers permanent anti-tumor immunity. In an embodiment, the CAR and one or more ETPs are transiently co-expressed in a T cell. Thus, the engineered T cells are safe (given the transient nature of the CAR/ETP expression), and induce prolonged (even permanent) immunity via APC function. As such, the T cells can be used to treat a wide variety of diseases associated with cell surface (target) antigens.

[0007] Accordingly, in one aspect, the invention provides a T cell comprising nucleic acid, e.g., exogenous nucleic acid, wherein

[0008] (a) the nucleic acid comprises a first nucleic acid sequence encoding a chimeric antigen receptor (CAR) comprising an extracellular domain, a transmembrane domain, and an intracellular signaling domain, and

[0009] (b) the nucleic acid comprises a second nucleic acid sequence encoding a polypeptide which enhances T cell priming, or a functional fragment or variant thereof,

provided that (i) the first and/or second nucleic acid sequence comprises an RNA; or

[0010] (ii) the CAR further comprises a second intracellular signaling domain, e.g., a costimulatory signaling domain. In an embodiment, the first and second nucleic acid sequences are disposed on a single nucleic acid molecule. In an embodiment, the nucleic acid molecule comprises RNA. In an embodiment, the nucleic acid molecule comprises DNA.

[0011] In an embodiment, the first and second nucleic acid sequences are disposed on two or more distinct nucleic acid molecules. In an embodiment, one or both nucleic acid molecules comprise RNA molecules. In an embodiment, one or both nucleic acid molecules comprise DNA molecules.

[0012] In an embodiment, one nucleic acid molecule comprises an RNA molecule and the other nucleic acid comprises a DNA molecule.

[0013] In an embodiment, the second intracellular signaling domain comprises a costimulatory signaling domain.

[0014] In an embodiment, the CAR comprises one or more costimulatory signaling domains.

[0015] In an embodiment, the intracellular signaling domain comprises a CD3zeta domain and the second intracellular signaling domain comprises a 4-1BB domain.

[0016] In an embodiment, the first nucleic acid sequence comprises an RNA. In an embodiment, the second nucleic acid sequence comprises an RNA. In an embodiment, the first and the second nucleic acid sequence each comprise RNA.

[0017] In an embodiment, the T cell is transfected to transiently express the first and/or second RNAs.

[0018] In an embodiment, the T cell does not comprise an exogenous DNA encoding the first or second RNA.

[0019] In an embodiment, the first and/or second RNAs are generated by in vitro transcription.

[0020] In an embodiment, the first and/or second RNAs are synthetic RNAs.

[0021] In an embodiment, the first and/or second RNAs are introduced into the T cell by electroporation.